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Published in:
Human Reproduction

DOI:
10.1093/humrep/des222

Human Reproduction, Vol.27, No.9 pp. 2850–2856, 2012
Advanced Access publication on June 27, 2012 doi:10.1093/humrep/des222

human reproduction

ORIGINAL ARTICLE ***Reproductive genetics***

Chromosomal abnormalities in azoospermic and non-azoospermic infertile men: numbers needed to be screened to prevent adverse pregnancy outcomes

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Submitted on January 18, 2012; resubmitted on April 19, 2012; accepted on May 21, 2012

STUDY QUESTION: How many infertile men who wish to conceive need to be screened for chromosomal abnormalities to prevent one miscarriage or the birth of one child with congenital anomalies (CAs)?

SUMMARY ANSWER: In azoospermic men, the prevalence of chromosomal abnormalities is 15.2% and the number needed to be screened (NNS; minimum–maximum estimate) for a miscarriage is 80–88 and for a child with CAs is 790–3951. The prevalence of chromosomal abnormalities in non-azoospermic men is 2.3% and the NNS are 315–347 and 2543–12 723, respectively.

WHAT IS KNOWN ALREADY: Guidelines advise the screening of infertile men for chromosomal abnormalities to prevent miscarriages and children with congenital abnormalities, but no studies have been published on the effectiveness of this screening strategy.

STUDY DESIGN, SIZE, DURATION: Retrospective cohort study of 1223 infertile men between 1994 and 2007.

PARTICIPANTS, SETTING, METHODS: Men with azoospermia and men eligible for ICSI treatment visiting a university hospital fertility clinic in The Netherlands who underwent chromosomal analysis between 1994 and 2007 were identified retrospectively in a registry. Only cases of which at least one sperm analysis was available were included. Data were collected by chart review, with a follow-up of pregnancies and their outcomes until 2010. The chromosomal abnormalities were categorized according to their risk of unbalanced offspring, i.e. miscarriage and/or child with CAs. Multi-level analysis was used to estimate the impact of chromosomal abnormalities on the outcome of pregnancies in the different subgroups of our cohort. NNS for miscarriages and children with CAs were calculated based on data from our cohort and data published in the literature.

MAIN RESULTS AND THE ROLE OF CHANCE: A chromosomal abnormality was found in 12 of 79 men with azoospermia (15.2%) and in 26 of 1144 non-azoospermic men (2.3%). The chromosomal abnormalities were categorized based on the literature, into abnormalities with and abnormalities without increased risk for miscarriage and/or child with CAs. In our study group, there was no statistically significant difference between the subgroups with and without increased risk respectively, regarding the frequency of children born with CAs (1/20; 5.0% versus 1/14; 7.1%), miscarriage (9/20; 45.0% versus 2/14; 14.3%) or unaffected liveborn children (9/20; 45.0% versus 9/14; 64.3%). The prevalence of chromosomal abnormalities with a theoretically increased risk of unbalanced progeny was 1.0% in non-azoospermic men and 3.8% in men with azoospermia. For the calculation of the NNS, the risk of an adverse pregnancy outcome in our cohort was compared with the incidence ranges of miscarriage and children with CAs in the general population. The number of azoospermic men that needs to be screened to prevent one miscarriage (80–88) or one child with CAs (790–3951) was considerably lower compared with the NNS in the non-azoospermic group (315–347 and 2543–12 723, respectively).

LIMITATIONS, REASON FOR CAUTION: The prevalence of chromosomal abnormalities in infertile men is low, and although we included 1223 men, our conclusions are based on a small number (38) of abnormal karyotypes. As there are no large series on outcomes of pregnancies in infertile men with chromosomal abnormalities, our conclusions had to be partly based on assumptions derived from the literature.

WIDER IMPLICATIONS OF THE FINDINGS: Based on the NNS calculated in our study, screening for chromosomal abnormalities is recommended in all azoospermic men. In non-azoospermic infertile men, screening might be limited to men with an additional risk factor (e.g. a history of recurrent miscarriage or a positive family history for recurrent miscarriage or children with CAs). The NNS can be used in future cost-effectiveness studies and the evaluation of current guidelines on karyotyping infertile men.

STUDY FUNDING/COMPETING INTEREST(S): Our institution has received research grants from Merck Sharpe & Dohme BV, Ferring Pharmaceuticals and Merck Serono, The Netherlands. No competing interests declared.

Key words: chromosomal abnormalities / miscarriage / congenital anomalies / male infertility

Introduction

The prevalence of chromosomal abnormalities and submicroscopic deletions of the Y chromosomal azoospermia factor (AZF) region is increased in infertile men (summarized by O'Flynn O'Brien *et al.*, 2010). Because of this increased prevalence, guidelines recommend screening for these genetic abnormalities before ICSI treatment in case of (severe) male infertility (NVOG, 1999; Crosignani and Rubin, 2000; Foresta *et al.*, 2002; NICE, 2004; AUA and ASRM, 2006). However, these guidelines are not based on clinical data and cost-effectiveness studies. So far, no studies have been published on the prevalence of adverse pregnancy outcomes in large cohorts of infertile men with genetic abnormalities.

The detection of a chromosomal abnormality in men with poor sperm quality allows clinicians to counsel the couple, not only regarding their chance of achieving a viable pregnancy with or without assisted reproductive technology (ART), but also on recurrent miscarriages or the risk of a child with congenital anomalies (CAs). The association between the latter two and balanced chromosomal rearrangements in particular, is well established (Tharapel *et al.*, 1985; Egozcue *et al.*, 2000; Suzumori and Sugiura-Ogasawara, 2010). The risk for a couple of having a child with an unbalanced karyotype depends on the segregation behaviour of the derivative chromosomes during meiosis and the size and nature of the translocated or inverted chromosome segments.

In a previous study, we found a low prevalence of chromosomal abnormalities in a cohort of 1223 men who were azoospermic or eligible for ICSI treatment (3.1%) (Dul *et al.*, 2010). There was a significant difference in the prevalence of chromosomal abnormalities between azoospermic men (15.2%) and non-azoospermic men (2.3%) (Dul *et al.*, 2012). The question whether or not to perform chromosomal analysis in infertile men not only depends on the prevalence of chromosomal abnormalities, but also on the consequences of these abnormalities. To explore the consequences, we addressed two questions in the present study: (i) Was ART performed in couples in which the male partner carried a chromosomal abnormality and what were the outcomes? (ii) Does the detected chromosomal abnormality have clinical relevance for the progeny of the couple and what would be the consequences if the abnormality had remained undetected? The aim of this study was to assess, by answering these questions for azoospermic and non-azoospermic men separately, the efficiency of screening infertile men for chromosomal abnormalities in terms of estimating the risk of an adverse pregnancy outcome. Therefore, we calculated the numbers needed to be screened (NNS) to have the option to prevent a miscarriage or the birth of a child with CA.

Materials and Methods

Our cohort consisted of men with azoospermia or eligibility for ICSI treatment who visited the fertility clinic of the University Medical Center Groningen between November 1994 and October 2007. Azoospermia and non-azoospermia were determined based on the first sperm analysis in our centre, as described in detail previously (Dul *et al.*, 2010). In 1223 men, the results of chromosomal analysis were available. Data on ART, ART related and spontaneous pregnancies (with a follow-up until 2010) and their outcomes were collected by chart review. In the study period, only ART with ejaculated sperm was offered in our centre.

No ethical board approval is required for retrospective chart review and collection of anonymized data in The Netherlands. Infertile couples attending our clinic are informed at intake about possible use of their anonymized data for research purposes, and a 'no objection procedure' is followed. Only patients who had not objected participated in this study.

Chromosomal analysis

Chromosomal analysis was performed on cultured peripheral lymphocytes. Five GTG-banded metaphase spreads with a minimal banding resolution of 550 were analysed per patient. In case of numerical mosaics, 100 metaphases were examined by conventional microscopic screening or FISH. When structural chromosomal aberrations were present, the evaluation was extended to additional molecular cytogenetic analysis by FISH or array comparative genomic hybridization, whenever appropriate. Chromosomal heteromorphisms as defined in the International System for human Cytogenetic Nomenclature 2009 were not considered chromosomal abnormalities (Shaffer *et al.*, 2009).

Classification of consequences

For each abnormality found, we performed a literature search for the risk of transmitting a chromosomal imbalance to the progeny. The estimation of the risk of unbalanced offspring is based on family history, and on published data on the expected segregation pattern of the translocation chromosomes and viability of the respective deletions and duplications (Stengel-Rutkowski *et al.*, 1988; Schinzel, 2001; Feenstra *et al.*, 2006). Based on these publications, we categorized the detected chromosomal abnormalities into the following categories: Chromosomal abnormality that does not increase the risk for miscarriage or a child with congenital anomalies not increased (NI); Chromosomal abnormality that increases

the risk of miscarriage (M); Chromosomal abnormality that increases the risk of both miscarriage and a child with CAs (M and CA). The incidences of miscarriages and of children with CA were compared between the latter two categories combined (M + M and CA) versus the NI group.

Calculation of NNS and statistical analysis

The NNS represents the number of subjects to be tested to detect one with a chromosomal abnormality that increases the risk for adverse pregnancy outcomes. For calculating NNS, the absolute difference in the risk of an adverse event in the study group and the risk in a reference population is calculated and the inverse of this risk difference is given as the NNS.

In order to calculate the NNS to prevent one miscarriage in infertile men, we first determined the incidence of miscarriage in the couples with a male karyotype known to be associated with an increased risk of miscarriage. However, not all miscarriages in this group will be due to an unbalanced karyotype and thus a correction needs to be made. This correction was based on the consistently reported incidence of clinically recognizable miscarriage in general population studies (12–15%) (summarized in Regan and Rai, 2000). Thus, the minimum NNS can be calculated as 1 divided by the prevalence of chromosomal abnormalities known to be associated with an increased miscarriage risk times the incidence of miscarriages in the study group minus the minimal reported general population incidence of 12%. The maximum NNS will be 1 divided by the prevalence times the incidence of miscarriages in the study group minus the maximal reported population incidence of 15% (see Table I).

Table I Calculation of NNS for miscarriage (M) and for a child with CA.

Formula number needed to screen		
1: [prevalence of chromosomal abnormalities with increased risk (incidence of adverse pregnancy outcome in study cohort—incidence in reference population)]		
Input parameters		
Prevalence of chromosomal abnormalities with increased risk of congenital anomalies		
Infertile men		11/1223
Azoospermic men		2/79
Non-azoospermic men		9/1144
Theoretical incidence of child with congenital anomalies in men with a chromosomal abnormality with increased risk of a child with congenital anomalies		4–8%
Incidence of child with congenital anomalies in general population		3%
Prevalence of chromosomal abnormalities with increased risk for miscarriage		
Infertile men		14/1223
Azoospermic men		3/79
Non-azoospermic men		11/1144
Incidence of miscarriage in men with a chromosomal abnormality with increased risk for M		45%
Incidence of miscarriage in reference population		12–15%
Number needed to screen for congenital anomalies		
Infertile men	1/[11/1223 × (0.08 − 0.03)] to 1/[11/1223 × (0.04–0.03)]	2224–11 123
Azoospermic men	1/[2/79 × (0.08 − 0.03)] to 1/[2/79 × (0.04 − 0.03)]	790–3951
Non-azoospermic men	1/[9/1144 × (0.08 − 0.03)] to 1/[9/1144 × (0.04 − 0.03)]	2543–12 723
Number needed to screen for miscarriage		
Infertile men	1/[14/1223 × (0.45 − 0.12)] to 1/[14/1223 (0.45 − 0.15)]	265–291
Azoospermic men	1/: [3/79 × (0.45 − 0.12)] to 1/[3/79 (0.45 − 0.15)]	80–88
Non-azoospermic men	1/[11/1144 × (0.45 − 0.12)] to 1/[11/1144 × (0.45 − 0.15)]	315–347

In order to calculate the NNS to prevent one child with CA, we used a different strategy as the number of pregnancies in the couples with a male chromosomal abnormality with an increased risk for a child with CA was too small to reliably determine the incidence of children with CA. Instead, we used the (theoretical) risk estimation for male carriers of a balanced chromosomal abnormality with a known increased risk of viable offspring with an unbalanced karyotype. In general, these risk figures may vary from as low as 1% to well above 20%, depending on the particular translocation, but the majority have a risk far below 5% (Stengel-Rutkowski *et al.*, 1988; Gardner and Sutherland, 2004). We conservatively estimated the risk of a child with CA to be increased by 1–5% for the carriers of a translocation with an assumed increased risk for CA. The estimated risk of 1–5% is the extra risk due to the paternal chromosomal abnormality and is added to the population risk of 3% of a child with CA. The minimum NNS for a child with CA thus will be 1 divided by the prevalence of chromosomal abnormalities related to an increased risk for CA times 5% and the maximum NNS as 1 divided by the prevalence times 1% (see Table I).

Since the prevalence of chromosomal abnormalities in men with azoospermia was significantly higher than in men with non-azoospermia, we calculated the NNS for both subgroups separately.

Statistical analysis was carried out using the Statistical Package for the Social Sciences version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were described in absolute counts and proportions. A generalized estimating equation analysis was used to estimate the impact of chromosomal abnormalities on the outcome of pregnancies in our cohort, accounting for the multi-level structure of the data, i.e. multiple pregnancies in the same couple. As 3 of 31 pregnancies in our cohort were twin pregnancies, we analysed the pregnancy outcomes per gestational sac (GS). Effects with a P-value < 0.05 were considered statistically significant.

Results

A chromosomal abnormality was detected in 38 of the 1223 men. The chromosomal abnormalities found are listed per sperm concentration category in Table II. In this table, the detected abnormalities are categorized according to their presumed consequences for progeny.

Table III summarizes the ART procedures and outcomes of pregnancies in our cohort of couples with a male chromosomal abnormality. In 21 of 26 couples with non-azoospermia, ART was started and 17 conceived. In two couples, a spontaneous pregnancy occurred. Azoospermic men with a gonosomal abnormality ($n = 10$) could not be offered ART in our clinic and these couples did not conceive. The remaining two azoospermic men [one with a reciprocal translocation (3;16) and one with a Robertsonian translocation (13;14) in combination with an inversion of chromosome 5] showed some spermatozoa in subsequent sperm analyses and ICSI treatment was started. The first couple did not conceive, but the latter did, which resulted in the birth of a healthy child.

Table II Chromosomal abnormalities per sperm concentration category found in a cohort of 1223 infertile men, categorized according to presumed consequences for the offspring [Partially adapted from Dul *et al.* (2010)].

Abnormality type per concentration category	Chromosomal abnormality	Prevalence per concentration category	Consequences for offspring[a]
Azoospermia		15.2% (12/79)	
Gonosomal	47,XXY (4 cases)		NI
	Mos 47,XXY [3]/46,XY [196]		NI
	Mos 45,X/46,X,der(Y).ish r(Y)(cp923.1-,SRY+,DYZ4+,DYZ3+)		NI
	Mos 45,X/46,X,idic(Y)(q11.2)		NI
	46,XX.ish(X)(SRY+)		NI
	46,X,der(Y)(pter ≥ q11.223::p11 ≥ pter)		NI
	46,X,t(Y;18;20)(q11.2;q12.2;q13.3)		M and CA
Translocation	46,XY,t(3;16)(q12;q23)		M
Translocation and inversion	45,XY,inv(5)(p13.1q13.1),der(13;14)(q10;q10)		M and CA
0–1 million/ml		3.1% (10/319)	
Gonosomal	47,XXY		NI
	47,XYY (2 cases)		NI
	Mos 47,XYY/46,XY		NI
Translocation	46,XY,t(1;14)(q44;q11.2)		M and CA
	45,XY,der(13,14)(q10;q10)		M and CA
Inversion	46,XY,inv(1)(p13q21)		NI
	46,XY,inv(2)(p11.2q13)		NI
	46,XY,inv(3)(q12q23)		NI
	46,XY,inv(11)(q21q23.3)		NI
1–5 million/ml		1.2% (3/251)	
Gonosomal	47,XYY		NI
	Mos 47,XXY [2]/46,XY [17]		NI
Inversion	46,XY,inv(10)(p11.2q21.2)		M
5–10 million/ml		1.4% (3/211)	
Translocation	46,XY,t(4:5)(q32;q14)		M
	46,XY,t(15;21)(q24;q22.3)		M and CA
	45,XY,der(13;14)(q10;q10)		M and CA
10–20 million/ml		3.1% (6/191)	
Gonosomal	Mos 45,X [4]/46,XY [26]		NI
	Mos 45,X [3]/46,XY [27]		NI
	Mos 45,X [4]/46,XY [116]		NI
Translocation	46,XY,t(3;11)(p21.3;q13)		M and CA
	45,XY,dic(13;14)(p11.2;p11.2)		M and CA
	45,XY,der(14;21)(q10,q10)		M and CA
>20 million/ml		2.3% (4/172)	
Translocation	46,XY,t(2;9)(q37.3;q12)		M and CA
	45,XY,der(15;21)(q10;q10)		M and CA
Inversion	46,XY,inv(2)(p21q14.2)		NI
	46,XY,inv(11)(q21q23.3)		NI

[*n*] represents number of cells within mosaic with that karyotype.
[a]NI: Chromosomal abnormality without increased risk for miscarriage or a child with congenital anomalies; M: Chromosomal abnormality with increased risk of miscarriage only; M and CA: Chromosomal abnormality with increased risk of miscarriage and child with congenital anomalies.

Table III Pregnancy outcomes in 38 couples in which the male partner had a chromosomal abnormality, categorized to its association with miscarriage and offspring with CAs.

	Total	%	NI	%	M + M and CA	%
Number of couples	38		24		14 (3 + 11)	
Of which treated	23	60.5	12	50.0	11 (3 + 8)	78.6
Total number of pregnancies (include spontaneous)	31		12		19 (2 + 17)	
Number of gestational sacs	34		14		20 (2 + 18)	
Live born normal (% per gestational sac)	18/34	52.9	9/14	64.3	9/20 (1 + 8)	45.0
Live born abnormal (% per gestational sac)	2/34	5.9	1/14	7.1	1/20 (0 + 1)	5.0
Miscarriage (% per gestational sac)	11/34	32.4	2/14	14.3	9/20 (0 + 9)	45.0
Ectopic pregnancy (% per gestational sac)	1/34	2.9	1/14	7.1	0/20 (0 + 0)	0
Unknown outcome of pregnancy (% per gestational sac)	2/34	5.9	1/14	7.1	1/20 (1 + 0)	5.0
At least one normal live born (% per treated couple)	12/23	52.2	6/12	50.0	6/11 (1/3 + 5/8)	54.5

NI: Chromosomal abnormality without increased risk for miscarriage or a child with congenital anomalies; M + M and CA: Sum of categories M: Chromosomal abnormality with increased risk of miscarriage only, and M and CA: Chromosomal abnormality with increased risk of miscarriage and child with congenital anomalies.

For our entire cohort of 1223 infertile men, the incidence of children with CA was 3.1% (38/1211). This incidence did not differ significantly from the incidence in 38 infertile couples with a male chromosomal abnormality, in which a child with CA was born to two couples (2/34; 5.9%). One was attributable to the paternal karyotype, but the other child had a normal karyotype, and the anomalies could not be related to the paternal chromosomal abnormality. Eleven of the 1223 men in our cohort carried a chromosomal abnormality that theoretically increases the risk of a child with CA by 1–5%. This is a conservative mean estimation based on a risk varying from <1% in translocation (13;14) carriers to >5% in the carrier of translocation (1;14). To determine the number of infertile men that need to be karyotyped to prevent one child with CA caused by a paternal chromosomal abnormality, we used the formula in Table I. The NNS in infertile men for one child with CA is 2224–11 123. In a population of men with azoospermia, this NNS is 790–3951 and in non-azoospermic men, 2543–12 723 (Fig. 1).

In our cohort, the incidence of a normal live born was 64.3% (9/14) in the NI group, and 45.0% (9/20) in the M + M and CA group. This difference was not statistically significant. If ART was performed, the chance for a couple of conceiving at least one normal live born was comparable in both groups (6/12; 47.4% and 6/11; 54.5%, respectively).

Six couples had one or more miscarriages. The incidence of miscarriages was higher in the M + M and CA group (9/20; 45.0%) compared with the NI group (2/14; 14.3%; $P = 0.052$). The incidence of miscarriages in both groups was not statistically different from the incidence of miscarriages in the men in our cohort with a normal karyotype, which was 25.1% (304/1211) (data not shown). To determine the number of infertile men that need to be karyotyped to find one miscarriage caused by a paternal chromosomal abnormality, we compared the miscarriage rate of our M + M and CA couples (45.0%) to the miscarriage rate in the general population (12–15%) (see Table I). The NNS in infertile men for a miscarriage is 265–291. In men with azoospermia the NNS is 80–88 and in non-azoospermic men, 315–347 (Fig. 1).

Discussion

The aim of this study was to assess the efficiency of screening infertile men for genetic abnormalities in terms of estimating the risk of an adverse pregnancy outcome (i.e. a child with CAs or a miscarriage). These data are urgently needed as a basis for evidence-based guidelines on screening. Our findings are based on a large cohort of 1223 infertile men of whom 38 (3.1%) carried a chromosomal abnormality. This relatively low prevalence is in agreement with reports from the literature. However, no data are published on pregnancy outcomes in large cohorts of infertile men with chromosomal abnormalities. We collected these data and calculated the NNS for adverse pregnancy outcomes. The NNS for miscarriage in men with azoospermia is 80–88 and for a child with CA 790–3951. In men with non-azoospermia the NNS for miscarriage is 315–347 and for a child with CA, 2543–12 723.

The most important consequence of genetic abnormalities in men with a procreative wish is the increased risk of a child with CA due to an unbalanced chromosomal abnormality. In the general population, the risk of a child with any CA is estimated to be 2–3%. Most gonosomal abnormalities may cause infertility in the offspring, but no other CA. The risk of CA is increased in translocations and some pericentric inversions, and the precise risk estimation is based on which chromosomes are involved and the breakpoints of the chromosomal anomaly. Worldwide published data on this risk estimation are used to counsel the couple on their specific risk. The estimation can be very precise when the abnormality is relatively common. However, for most abnormalities, no data for calculating the risk are available. We did not find a significant difference in the incidence of children with CA born to infertile men with and without chromosomal abnormalities. This is probably caused by the small number of pregnancies in our study in the men with chromosomal abnormalities. For the calculation of an NNS, we therefore used the theoretical risk of a child with CA in these men, based on the literature. Because in the study period only ART with ejaculated sperm could be offered in our clinic, only the couples with non-azoospermia have undergone ART and conceived. Based on the data obtained in our study, the NNS for a child with CA is 2543–12 723 in men with non-azoospermia. If ICSI with surgically retrieved sperm is performed in azoospermic men with genetic abnormalities, the risk for

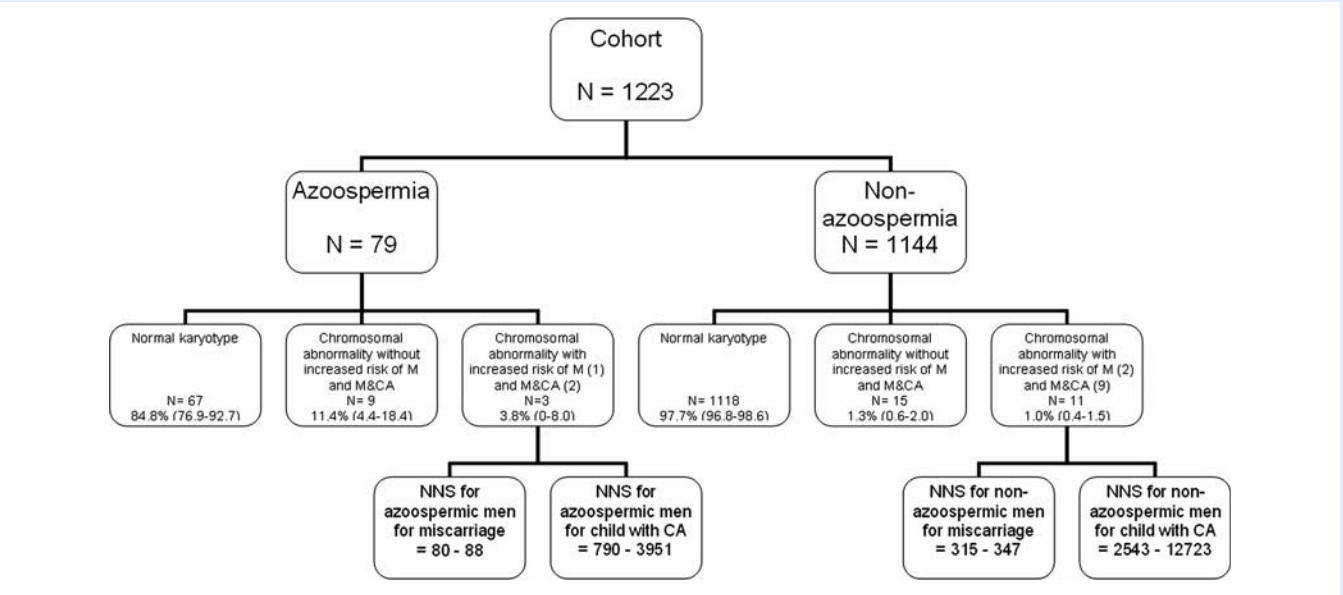

Figure 1 Prevalence (95% confidence intervals) of chromosomal abnormalities and NNS for miscarriage (M) and child with CAs in a cohort of infertile men.

the offspring is expected to remain low since most genetic abnormalities found in azoospermic men are not associated with miscarriage or children with CA. If we extrapolate the results of this study to men with azoospermia, the NNS for a child with CA in azoospermic men would be 790–3951.

Another consequence of chromosomal abnormalities can be an increased risk (in reviews varying from 49 to 83%) of miscarriages, especially in translocations and pericentric inversions (Franssen *et al.*, 2006; Stephenson and Sierra, 2006; Suzumori and Sugiura-Ogasawara, 2010). In our study, ART treatment of non-azoospermic men with chromosomal anomalies did not result in significantly more miscarriages (32.4%), when compared with men with a normal karyotype (25.1%). Based on our data, the NNS for miscarriage in infertile men is 265–291 (80–88 for azoospermic men and 315–347 for non-azoospermic men). In non-azoospermic men, it might be more cost-effective to limit screening for chromosomal abnormalities to those with a history of more than one miscarriage. In recurrent miscarriage, the risk that one of the partners of a couple is a carrier of a translocation or inversion is 4–6% (Goddijn and Leschot, 2000), while in our study the prevalence of chromosomal abnormalities with an increased risk of miscarriages was only 1.0% for the male partner with non-azoospermia. A higher prevalence of structural chromosomal abnormalities, as in couples with recurrent miscarriages, might lower the NNS substantially. The low prevalence of translocations and inversions among infertile men indicates that male infertility alone is not the most effective selection criterion for genetic screening. It remains to be established whether a positive history for recurrent miscarriage or a positive family history for recurrent miscarriage or children with CA decreases the NNS significantly in non-azoospermic infertile men. Research on the impact of recurrent miscarriages in relatives on the NNS might be hampered by non-disclosure of miscarriages, which may be culturally determined.

Most non-azoospermic men in our cohort with an increased risk of unbalanced progeny did not refrain from ART after counselling, and conceived. This did not result in a significantly higher incidence of miscarriages or offspring with CAs when compared with infertile men with a chromosomal abnormality without a known increased risk for adverse pregnancy outcome. This may indicate that screening for chromosomal abnormalities in non-azoospermic men does not decrease the incidence of adverse pregnancy outcomes.

Screening for chromosomal abnormalities, and also for AZF deletions, in men with poor sperm quality may serve another goal: it might give an explanation for the infertility, as in some chromosomal abnormalities (e.g. Klinefelter's syndrome and Robertsonian translocations), and in AZF a and b deletions the association with infertility is well-known. A causative diagnosis may be helpful in the emotional coping process of the couple. Furthermore, in some countries, legislation prescribes testing for genetic causes in all infertile couples. This may include testing for cystic fibrosis-related and androgen receptor gene mutations, as well as other gene mutations and polymorphisms suspected to be involved in infertility (O'Flynn O'Brien *et al.*, 2010). Most guidelines advise the screening of infertile men for chromosomal abnormalities and AZF deletions and the performance of elaborate testing based on the clinical presentation. We explored the efficiency of the common policy of screening for chromosomal abnormalities and AZF deletions, in terms of finding the cause of the male infertility (Supplementary data, Table SI). We found that most genetic abnormalities detected in azoospermic men had a known association with infertility, while the majority of genetic abnormalities found in non-azoospermic men were chance findings (i.e. those occurring with a low frequency in the population and unrelated to the infertility of the couple). In our study, azoospermia was most frequent in men with gonosomal abnormalities (including AZF deletions) and Robertsonian translocations, while the majority of men with reciprocal

translocations and inversions were non-azoospermic. This is in agreement with the literature (reviewed in O'Flynn O'Brien *et al.*, 2010).

For the development of evidence-based guidelines on the genetic screening of infertile men cost-effectiveness, analyses have to be performed that also consider the costs of adverse outcomes of pregnancies, and the costs of prevention of miscarriages and children with CAs by preimplantation genetic diagnosis and prenatal diagnosis. The current study provides data on the NNS for these adverse outcomes, to be used in future cost-effectiveness studies. The NNS in men with azoospermia for miscarriage (80–88) and for a child with CA (790–3951) are relatively low compared with men with non-azoospermia, in whom the NNS for miscarriage is 315–347 and the NNS for a child with CA is 2543–12 723. The large difference in these NNS between men with azoospermia and non-azoospermia is caused by the significant difference in the prevalence of chromosomal abnormalities in these two groups of infertile men. The probability of finding a chromosomal abnormality is even higher in men with non-obstructive azoospermia compared with men with obstructive azoospermia (O'Flynn O'Brien *et al.*, 2010). As a consequence, the NNS in men with non-obstructive azoospermia will be substantially lower. Due to the retrospective design of our study, this subcategorization of azoospermia was not possible. Future studies should address this issue, to allow for a better risk estimate in obstructive and non-obstructive azoospermia.

Based on the NNS found in our study, we recommend performing chromosomal screening in all azoospermic men, because a relatively low number of men need to be screened to prevent adverse pregnancy outcomes. In non-azoospermic infertile men, screening might be limited to men with a history of recurrent miscarriage or with a positive family history for recurrent miscarriage or children with CAs. Future cost-effectiveness studies are needed to provide further evidence that will aid in the development of guidelines on screening infertile men for chromosomal abnormalities.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors' roles

E.C.D. helped design the study, collected and analyzed the data and wrote the manuscript. J.v.E.-A. was responsible for the sperm analyses. H.G. supervised the statistical analysis. T.D. was responsible for the chromosomal analyses. J.A.L. was responsible for the design of the study. C.M.A.v.R.-A. helped design the study and provided the cytogenetic data. All authors revised the manuscript.

Funding

This study was supported by research grants from Merck Sharpe & Dohme BV, Ferring Pharmaceuticals, and Merck Serono, The Netherlands.

Conflict of interest

None declared.

References

AUA and ASRM: American Urological Association and American Society for Reproductive Medicine. Report on optimal evaluation of the infertile male. *Fertil Steril* 2006;**86**:S202–S209.

Crosignani PG, Rubin BL. Optimal use of infertility diagnostic tests and treatments. The ESHRE Capri Workshop Group. *Hum Reprod* 2000;**15**:723–732.

Dul EC, van Ravenswaaij-Arts CMA, Groen H, van Echten-Arends J, Land JA. Who should be screened for chromosomal abnormalities before ICSI treatment? *Hum Reprod* 2010;**25**:2673–2677.

Dul EC, Groen H, van Ravenswaaij-Arts CMA, Dijkhuizen T, van Echten-Arends J, Land JA. The prevalence of chromosomal abnormalities in subgroups of infertile men. *Hum Reprod* 2012;**27**:36–43.

Egozcue S, Blanco J, Vendrell JM, Garcia F, Veiga A, Aran B, Barri PN, Vidal F, Egozcue J. Human male infertility: chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion. *Hum Reprod Update* 2000;**6**:93–105.

Feenstra I, Fang J, Koolen DA, Siezen A, Evans C, Winter RM, Lees MM, Riegel M, de Vries BBA, Van Ravenswaaij CMA *et al.* European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA) an online database for rare chromosome abnormalities. *Eur J Med Genet* 2006;**49**:279–291.

Foresta C, Ferlin A, Gianaroli L, Dallapiccola B. Guidelines for the appropriate use of genetic tests in infertile couples. *Eur J Hum Genet* 2002;**10**:303–312.

Franssen MT, Korevaar JC, van der Veen F, Leschot NJ, Bossuyt PM, Goddijn M. Reproductive outcome after chromosome analysis in couples with two or more miscarriages: index [corrected]-control study. *Br Med J* 2006;**332**:759–763.

Gardner RJM, Sutherland GR. *Chromosome Abnormalities ad Genetic Counseling*, 3rd edn. Oxford, UK: Oxford University Press, 2004.

Goddijn M, Leschot NJ. Genetic aspects of miscarriage. *Baillieres Best Pract Res Clin Obstet Gynaecol* 2000;**14**:855–865.

NICE: National Institute for Clinical Excellence. *Fertility: Assessment and Treatment for People with Fertility Problems*. London: RCOG Press, 2004. Available on: http://www.nice.org.uk/nicemedia/live/10936/29269/29269.pdf (18 January 2012, date last accessed).

NVOG (Dutch Society of Obstetrics and Gynaecology). *Guideline: Assessment and Treatment for Male Subfertility*, 1999. NVOG-richtlijn 17:1–5. Available on (in Dutch): http://nvog-documenten.nl/uploaded/docs/17_onder_behan_mannesub.pdf (7 September 2010, date last accessed).

O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. *Fertil Steril* 2010;**93**:1–12.

Regan L, Rai R. Epidemiology and the medical causes of miscarriage. *Baillieres Best Pract Res Clin Obstet Gynaecol* 2000;**14**:839–854.

Schinzel A. *Catalogue of Unbalanced Chromosome Aberrations in Man*, 2nd edn. Berlin, Germany: De Gruyter, 2001.

Shaffer LG, Slovak ML, Campbell LJ. *ISCN 2009: An International System for Human Genetic Nomenclature*. 1st edn. Basel, Switzerland: Karger Publishers, 2009.

Stengel-Rutkowski S, Stene J, Gallano P. Risk estimates in balanced parental reciprocal translocations. *Monographie des Annales de Génétique* 1988;**14**:1–147.

Stephenson M, Sierra S. Reproductive outcomes in recurrent pregnancy loss associated with a parental carrier of a structural chromosome rearrangement. *Hum reprod* 2006;**21**:1076–1082.

Suzumori N, Sugiura-Ogasawara M. Genetic factors as a cause of miscarriage. *Curr Med Chem* 2010;**17**:3431–3437.

Tharapel AT, Tharapel SA, Bannerman RM. Recurrent pregnancy losses and parental chromosome abnormalities: a review. *Br J Obstet Gynaecol* 1985;**92**:899–914.